Abstract

Muscle development and function are highly synchronized processes that involve simultaneous action of several transcription factors, signalling cascades, kinases and phosphatases, ion channels, structural proteins and secondary messengers. Several aspects of muscle biology including contraction, development, growth, regeneration etc. have been extensively studied using model organisms like *Mus musculus, C. elegans, Drosophila melanogaster, Danio rerio* and *Xenopus*. **Chapter 1** provides an in-detail account of work done previously in the field of muscle biology that includes literature about different types of muscles in vertebrates, their localization and function in the body, arrangement of thick and thin filaments in different types of muscles and their development. Of the several model organisms available, *Drosophila* has long been a favored model organism for muscle biologists. The indirect flight muscles (IFMs) of *Drosophila* are structurally similar to vertebrate skeletal muscles and physiologically to cardiac muscles and have been extensively used as a model to study vertebrate muscle development and function. Chapter 1 provides an overview of literature on development and function of IFMs in *Drosophila*. An essential and probably most important second messenger utilized by muscles for their function is Calcium. Muscles harbor an intricate and elaborate machinery of proteins that can sense calcium, transport calcium in and out of the cell, bind calcium and regulate gene expression and so on. **Chapter 1** briefly explains the studies done previously with respect to calcium signalling in muscles. Background, which led to hypotheses for present study, has been described in the chapter. **Chapter 2** includes details about all the techniques that have been used to conduct the experiments.

Calcium signalling plays an important role not just in functioning of muscles but also in development, growth, injury and regeneration of the tissue. Cells have an elaborate machinery of calcium handling proteins that work in synchrony to achieve a desired state of function. Details of this have been included in **Chapter 4**. Calcium handling machinery is comprised of ion channels like voltage gated calcium channels, calcium activated potassium channels, calcium binding proteins, structural proteins etc. **Chapter 3** talks about one of the calcium binding protein, Calcineurin, whose function has been implicated in fibre type switching in vertebrate muscles. Its expression in the muscles increases during exercise or weightlifting suggesting its role in conversion of fast glycolytic fibres to slow oxidative fibres that are required for maintaining sustained force in the muscles. Calcineurin works in concert with several transcription factors to bring about changes in transcription under conditions of stress. It is known to interact with NFAT transcription factor to regulate fibre type switching. It is also known to work along with Mef2 transcription factor to regulate expression of genes. Current study shows that the reduction in levels of Calcineurin-A subunit in muscles does not affect function or structure of muscles, but over-expression of the protein causes premature death of majority of the organisms and flight lessness in the escaper flies. On the contrary, loss of regulatory subunit of the protein, Calcineurin-B2, causes muscle hypercontraction in IFMs of *Drosophila*, suggesting crucial role of the protein in IFM development. The slow, progressive degeneration of IFMs in *calcineurin-B2 (*

canB2) mutants is reminiscent of the muscle hypercontracted phenotype observed in mutants of Myosin heavy chain and the Troponin T and Troponin I proteins. Genetic studies of calcineurin with mutants of Troponin T and I show a synergistic interaction between Troponin T mutant *up101* and *calcineurin-B2*. The *Drosophila* Troponin T mutation*, up101*, is equivalent to human cardiomyopathy Troponin T mutations, R92Q and I79N. The contractile

machinery of the Troponin T mutants shows increased sensitivity towards calcium and can contract at the calcium concentrations below the threshold level. Current study **(Chapter 3)** highlights the importance of calcineurin in maintaining calcium homeostasis in muscles. Loss of calcineurin leads to arrhythmic spontaneous calcium oscillations in IFMs, which means that the average time for which the contraction machinery remains in contact with calcium is higher in *canB2* mutant than control (frequency of oscillations is higher in *canB2* mutants than control) and this probably contributes to the enhanced hypercontraction phenotype in a calcium sensitive mutant of Troponin T. Arrythmicity in the calcium oscillations is observed as early as 50hrs after puparium formation (APF), well before the muscle degeneration phenotype is manifested in *canB2* mutant flies. This reflects towards the importance of calcineurin in maintenance of calcium homeostasis in muscles.

Chapter 4 describes study of spontaneous calcium oscillations during IFM development. Calcium is a highly versatile signal that works at different time points to regulate several cellular processes. Spontaneous calcium oscillations have been extensively studied in striated muscles, both the cardiac and skeletal muscles. There are different types of oscillations to which muscles respond. Long duration calcium transients (LDTs) have been identified in Xenopus myocytes and they predominantly occur prior to myofibrillogenesis, whereas SDTs (Short duration calcium transients) are spontaneous calcium oscillations of short duration (2- 3sec) that originate in subsarcolemmal space and are ryanodine sensitive, insensitive to changes in membrane potential and are independent of extracellular calcium. Similar to vertebrate muscles spontaneous calcium oscillations are also observed in IFMs of *Drosophila* throughout development. These oscillations were not reported previously in this system. We observe that the calcium oscillations in IFMs start as early as 34hrs APF, coinciding with the initiation of myofibrillogenesis process. There were no evident oscillations before 34hrs APF (i.e. from 0- 34hrs; the time point that involves processes of muscle splitting and myoblast fusion). The nature of these oscillations is still obscure.

These oscillations vary in frequency, peak width and peak area across development. Previous reports have shown that cells often respond to changes in stimulus by varying frequency of calcium waves. These frequency changes are decoded by sophisticated molecular machines that include calcium sensitive proteins like calcium/calmodulin dependent protein kinase II and protein kinase C. The difference in peak frequency observed in the developing IFM could be due to differential expression of ion channels and structural proteins at these stages. Indeed, our results show that channels like Ryanodine, STIM and cacophony are transcriptionally regulated, and their transcripts are expressed only in adults whereas transcripts of channels like SERCA and slowpoke (Calcium gated potassium channels) are detected strongly throughout the development.

Current study shows that spontaneous calcium oscillations in IFMs are sensitive to the levels of SERCA channels. These channels localize to the endoplasmic reticulum and are required for transportation of calcium from cytosol to endoplasmic reticulum. SERCA calcium pump and its function of calcium sequestration is essential for both development and functioning of the muscles because majority of the animals with reduced expression of SERCA do not survive till adult stage, rather they die in early larval or early pupal stages. Knockdown of SERCA in IFMs leads to increase in peak area and peak width of the calcium oscillations, which suggests the defect in calcium sequestration ability of the muscles. This abnormality in the calcium quenching could result in irregular muscle contraction in adult flies, which is shown by the contracted state of the adult muscles in escaper flies.

Spontaneous oscillations are also sensitive to the changes in intracellular calcium levels. Reduction in the levels of intracellular calcium by over-expression of calcium binding protein, Parvalbumin, reduces the frequency of oscillations in developing IFM. These flies show defects in their flight ability suggesting that calcium is utmost important for the functioning of the muscles.

Taken together, these studies reflect upon importance of calcium signalling in muscle development and function.